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PHYSIOLOGICAL RESPONSE TO ATMOSPHERIC POLLUTANTS

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16. ABSTRACT

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Previous studies have shown that certain sulfates and sulfuric acid elicit greater response in the lung than comparable amounts of sulfur dioxide. There is therefore a need to develop information which will relate these findings to the concentration of pollutants found in ambient air. This project has performed this function.

John H. Knelson, M.D.
Director,
Health Effects Research Laboratory

ABSTRACT

During the period of this grant several materials were examined as air pollutants of interest for their irritant effects. These included sulfuric acid, a series of inorganic sulfates, and a combination of ozone and sulfur dioxide. Some attention was also given to the effect of various oil mists on the irritant response to sulfur dioxide. The method used for measuring irritant response was by simultaneous tracings of intrapleural pressure, tidal volume, and rate of flow of gas in and out of the respiratory system. By relating the intrapleural pressure change to the change in flow rate at points of equal lung volume, it was possible to calculate the flow resistance; by relating pressure change to volume at the beginning and end of inspiration, it was possible to calculate compliance. The concentrations used in these studies are well within the range of human exposure. These studies indicate that the irritant response previously observed at higher concentrations of sulfuric acid is also observed at concentrations below 1 mg/m^3. The failure of alterations in resistance to return promptly to control values following termination of exposure has been a consistent finding in the work with various irritant aerosols. The lowest concentrations used in these studies (100 $\mu g/m^3$) are in the range of concentrations which have been reported as short-term maxima in urban atmospheres. Data obtained by the methods used in these studies can be applied in concept (although not by direct extrapolation) to the response of sensitive individuals to short-term peaks of pollution.

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I. Scope of Work

During the period of this grant we have examined several materials of interest as air pollutants for their irritant effects. These have included sulfuric acid, a series of inorganic sulfates, and a combination of ozone and sulfur dioxide. Some support was also given to a project studying the effect of various oil mists on the irritant response to sulfur dioxide. Preliminary work was done with sulfite and bisulfite.

II. Physiological Methods

The method we use of measuring irritant response has been described in the literature (Amdur and Mead, Am. J. Phys. 192:364, 1958). Simultaneous tracings are needed of intrapleural pressure, tidal volume, and rate of flow of gas in and out of the respiratory system. Intrapleural pressure is measured by recording the pressure changes in a fluid-filled catheter which is inserted under ether anesthesia of brief duration. Tidal volume is measured by recording the pressure changes produced in a body plethysmograph. Rate of flow is measured by electrical differentiation of the volume signal with respect to time. By relating the intrapleural pressure change to the change in flow rate at points of equal lung volume, it is possible to calculate the flow resistance. By relating pressure change to volume at the beginning and end of inspiration, it is possible to calculate compliance.

The method has certain advantages. One of these is its simplicity, which permits routine toxicological use of unanesthetized animals. The fact that each animal serves as its own control per-

mits the use of paired data for statistical evaluation. The increase in flow-resistance is related to the concentration of irritant; this permits the development of dose-response curves. These curves yield information on such factors as the effect of particle size on the potency of an irritant material, on the relative irritant potency of different materials, and on the effect of inert particulate material on the degree of response to irritant gases. Data such as these dose-response curves resulting from a single exposure of many animals over a wide range of concentrations provide a tool for the demonstration of potentiation effects and other subtle toxicological phenomena which cannot readily be detected by experiments on human subjects. Experimentation with human subjects is by practical necessity limited both as to number of subjects and conditions of exposure.

The method also has certain limitations which should be kept clearly in mind. The standard exposure time is one hour; at best, the preparation can only be used for five or six hours. Thus, the results obtained cannot be used to predict effects of chronic exposure to the compounds studied. Although the animals are unanesthetized during the experimental period, an intrapleural catheter has been inserted under anesthesia. That this surgical procedure per se causes an increase in resistance was demonstrated by measurements made by another method before and after the insertion of the catheter (Mead, J. Appl. Physiol. 15:325, 1960). The physiological technique thus constitutes a stress which might render such animals more sensitive to irritant exposure. There is experimental evidence to support this suggestion. Some unpublished experiments in which the

response to sulfur dioxide was measured by another method that did not involve the use of an intrapleural catheter indicated that the two methods were of equivalent sensitivity at high concentrations but that at concentrations of 2 ppm and less the intrapleural catheter method was more sensitive. Thus the stress of the technique used has rendered the animals more sensitive to low-grade irritant exposure. This might be considered an advantage rather than a limitation, as it is the sensitive segment of the human population that is most affected by air pollution. In any effort, to draw meaningful extrapolations from these data, it should be kept in mind that if these guinea pigs are analogous to anything at all, it is to the sensitive individual and not to the normal healthy individual.

Random-bred guinea pigs weighing 200-300 g were used in these experiments. The plethysmograph was clamped so that the animal's head projected into the exposure chamber. Respiratory measurements were made every five minutes during a half-hour control period. The material or materials being studied were then added to the entering air stream of the exposure chamber. Respiratory measurements were again made every five minutes during an exposure period of one hour (for sulfuric acid or sulfate salts) or two hours (for the ozone-sulfur dioxide studies). Each animal thus served as its own control. The chamber was cleared of irritant material and measurements were made during a post-exposure period of one-half to one hour.

III. Sulfuric Acid

A. Method of Aerosol Generation and Measurement

The sulfuric acid aerosol is produced by a Rappaport-Weinstock condensation type aerosol generator (Rappaport and Weinstock, Experientia 11:363, 1955). The sulfuric acid is first heated and nebulized to a heterogeneous aerosol. The larger particles are removed by impaction and the smaller particles are carried upwards through a heating column in which they are vaporized. Upon emerging from the heating column, the vapor cools and condenses into droplets of uniform size. The size of the aerosol can be controlled by appropriate adjustments of the amount of sulfuric acid nebulized, of the temperature of the reservoir, and the temperature of the heating column. The mass concentrations can be controlled by regulating the amount of dilution air. All air is pre-dried and filtered to remove foreign particles. Relative humidity of the chamber was 50% and temperature was 70°F.

The mass concentration was measured by collecting a sample of the aerosol on a type G.S. Millipore Filter, which was then soaked in 10 ml demineralized water. The electrical conductivity of the resulting solution was then measured and compared with a standard curve. All samples were collected throughout the animal exposure period at a flow rate of 3.5 lpm. Concentrations were reproducible within ± 10% or better.

The particle size of the larger aerosol was determined before and after each animal exposure with an ultra-microscope by which the time of free fall of the aerosol particles across a calibrated grid could be measured. The size was then determined by appropri-

ately inserting the free fall time into the Stokes-Cunningham equation. Average sizes were based on counts of 50-100 particles. The standard deviation was consistently less than 10% of the mean diameter. The particle size of the smaller aerosol was determined by light scattering at each set of experimental conditions.

B. Background for Present Studies

Sulfuric acid is the most irritant of the particulate sulfur species formed by the oxidation of sulfur dioxide in the atmosphere. Acute exposure of guinea pigs has demonstrated that the irritant potency of a given amount of sulfur, as evaluated by changes in respiratory mechanics, may be increased three- to five-fold when given as sulfuric acid rather than as sulfur dioxide (Amdur, Am. Indust. Hyg. Assoc. J. 35:489, 1974). In two-year exposures of monkeys to sulfuric acid, pathological changes were produced which were not observed when corresponding levels of sulfur were given as sulfur dioxide (Alarie et al., Arch. Environ. Health 27:16, 1973). The greater irritant potency of sulfuric acid is thus indicated in the acute response of a rodent species and the chronic response of a primate species. Aerosols of metal salts, which promoted the conversion of sulfur dioxide to sulfuric acid, potentiated the response to sulfur dioxide three- to four-fold (Amdur and Underhill, Arch. Environ. Health 16:460, 1968).

Our previously-published study of the respiratory response of guinea pigs to sulfuric acid (Amdur, Arch. Indust. Health 18:407, 1958) did not examine concentrations below 2 mg/m^3 nor particle

sizes below 0.8 μm (MMD). The studies reported here were performed to obtain similar data on concentrations of 1 mg/m^3 and below at particle sizes of 1 and 0.3 μm.

C. Results

Table 1 presents our data on the effect of sulfuric acid on pulmonary flow resistance and pulmonary compliance. All exposures produced a statistically significant increase in resistance. The degree of response was dose-related as shown in Figure 1. The 0.3 μm particles produced a greater response at a given concentration than did the 1 μm particles. This difference appears greatest at the lowest concentration of 0.1 mg/m^3.

In animals exposed to 0.1 mg/m^3 at the 1 μm particle size, the resistance values had returned to pre-exposure values by the end of the half-hour post-exposure period. In all other exposures, the post-exposure value was less than the response at the end of exposure but was still elevated above control values. The time course of the post-exposure resistance increases, expressed as percent change from control, is shown in Figure 2 for the 0.3 μm particles at concentrations of 0.1 and 1 mg/m^3. In both cases, the values increased slightly during the first five minutes of the post-exposure period then declined to an essentially constant level by fifteen minutes after the end of exposure.

A decrease in pulmonary compliance was also produced by these low level exposures to sulfuric acid. In the exposure to 1 μm particles, the decrease in compliance was not statistically significant at concentrations of 0.1 or 0.4 mg/m^3. A decrease in compliance was produced by the two higher concentrations of 1 μm particles.

At a particle size of 0.3 μm, a decrease in compliance was observed at all concentrations tested. At corresponding concentrations, the 0.3 μm particles produced a greater decrease in compliance than the 1 μm particles. When the exposure produced a decrease in compliance, the values were still below control values at the end of the thirty-minute post-exposure period, although less depressed than at the end of exposure.

Detailed data are not presented for tidal volume, respiratory frequency, or minute volume, as the low concentrations used in these studies produced no alterations in any of these factors.

D. Discussion

The concentrations used in these studies are well within the range of human exposure. Concentrations above 0.1 mg/m^3 have been reported as hourly averages in urban pollution. A concentration of 1 mg/m^3 is the currently recommended standard for occupational exposure.

These studies indicate that the irritant response previously observed at higher concentrations of sulfuric acid is also observed at concentrations below 1 mg/m^3. The response to 0.1 mg/m^3 at the 1 μm particle size is slight and rapidly reversible. The response to 0.1 mg/m^3 at the 0.3 μm size is greater in magnitude and is not rapidly reversible.

A concentration of 1 mg/m^3 sulfuric acid contains 0.3 mg/m^3 of sulfur. The percent increase in resistance produced is on the order of 80% for 0.3 μm particles and 60% for 1 μm particles. The same order of magnitude of sulfur (0.2 mg/m^3) given as sulfur

dioxide (0.16 ppm) produces a slight resistance increase in the order of 10%. Thus, the same amount of sulfur, when given as sulfuric acid, produces 6 to 8 times the response observed when given as sulfur dioxide. Given as 1 μm sulfuric acid, 0.03 mg S/m^3 produces a response of the same order of magnitude as 0.2 mg S/m^3 given as sulfur dioxide. The response to 0.03 mg S/m^3 as 0.3 μm sulfuric acid is four times the response to 0.2 mg S/m^3 given as sulfur dioxide.

The failure of alterations in resistance to return promptly to control values following termination of exposure has been a consistent finding in our work with various irritant aerosols. The post-exposure resistance values of animals exposed to sulfur dioxide and sodium chloride or to formaldehyde and sodium chlcride remained elevated and were related to the total does of aerosol. This was one of the earlier findings which suggested that the potentiation of the response to these gases was mediated by formation of an irritant aerosol rather than by simple transfer of additional gas as such to the lung (Amdur, Inhaled Particles and Vapors I:281, 1961; Amdur and Underhill, Arch. Environ. Health 16:460, 1968). The response to sulfur dioxide or formaldehyde alone was readily reversible until extremely high concentrations were reached. The only exception to the slow reversibility of the response to irritant aerosols was histamine, with which even very major responses were almost immediately reversible (Amdur, Arch. Environ. Health 13:29, 1966).

The lowest concentrations used in these studies (100 $\mu g/m^3$) are in the range of concentrations which have been reported as short-term maxima in urban atmospheres. Data obtained by the methods used in these studies can be applied in concept (though obviously not by direct extrapolation) to the response of sensitive individuals to short-term peaks of pollution. Alteration of pulmonary mechanics is obviously only one manifestation of irritant response. Another manifestation is alteration in regional deposition or clearance of aerosols. It is of interest to note that alterations in regional deposition patterns have been demonstrated in guinea pigs exposed to concentrations as low as 30 $\mu g/m^3$ (size 0.25 μm) for one hour (Fairchild et al., Amer. Indust. Hyg. Assoc. J. 37:584, 1975). More recently, Dr. Morton Lippmann's group at New York University has found that a one hour exposure to < 200 $\mu g/m^3$ (size 0.3 μm) caused a significant transient slowing of tracheobronchial clearance of ferric oxide aerosol in donkeys. Thus, these levels of sulfuric acid produce irritant effects other than the alterations in pulmonary mechanics reported in the present studies.

Table 1

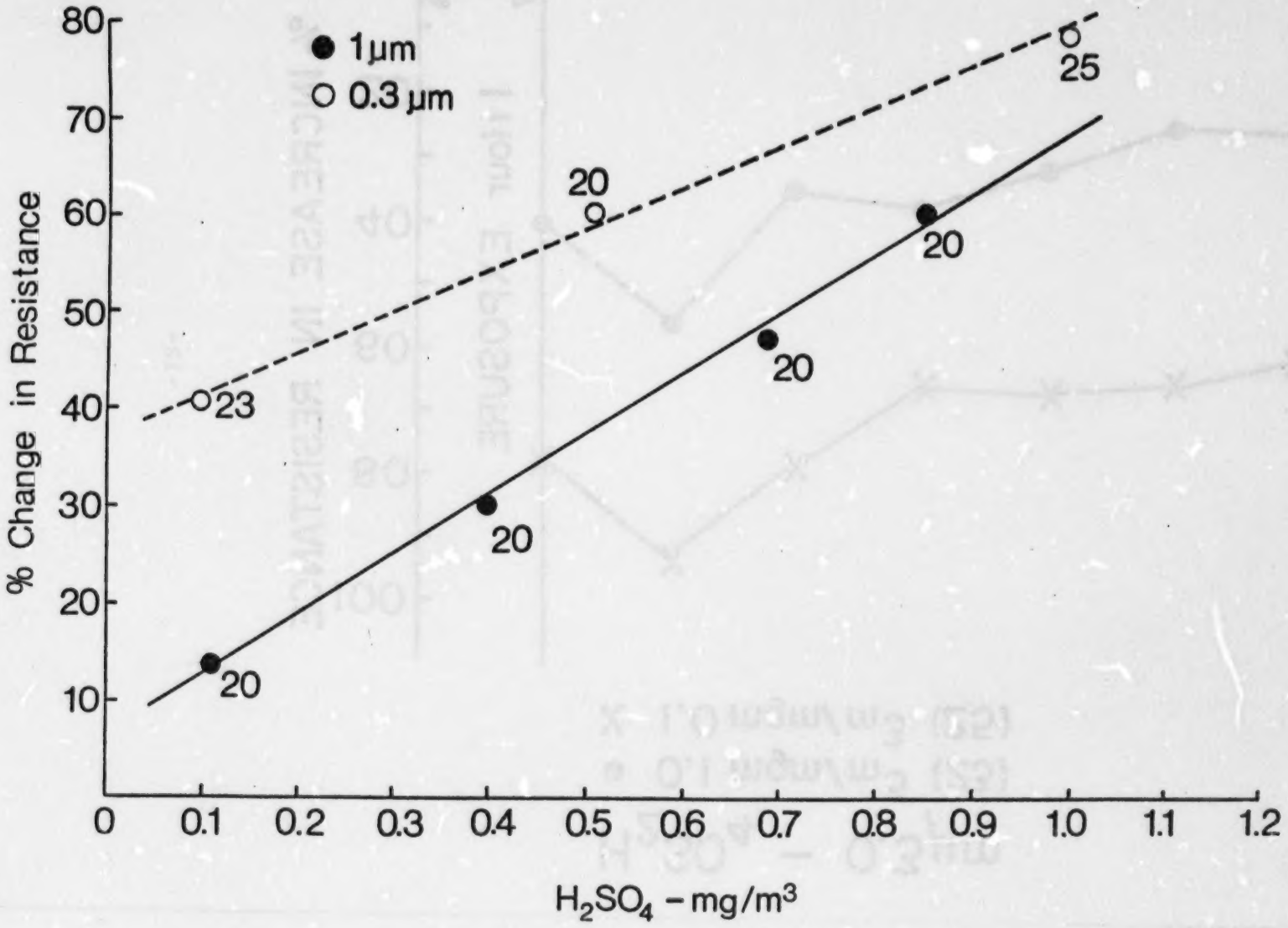
Respiratory Response to Sulfuric Acid

Particle Size MMD	1 µm				0.3 µm		
Concentration mg/m^3	0.11	0.40	0.69	0.85	0.10	0.51	1.0
Number of Animals	20	20	20	20	23	20	25
Resistance cm H_2O/ml/sec							
Control	0.70	0.59	0.72	0.68	0.80	0.72	0.74
Exposure[a]	0.80	0.77	1.06	1.09	1.13	1.15	1.32
$\bar{x}$ (E-C)	0.10	0.18	0.34	0.41	0.33	0.43	0.58
$S_{\bar{x}}$	0.039	0.033	0.042	0.051	0.048	0.052	0.063
$P^{\bar{x}}$ <	0.02	0.001	0.001	0.001	0.001	0.001	0.001
% Change	+14	+30	+47	+60	+41	+60	+78
Post Exposure[b]	0.77	0.71	0.89	0.86	1.01	1.09	1.20
$\bar{x}$ (P-C)	0.07	0.12	0.17	0.18	0.21	0.37	0.46
$S_{\bar{x}}$	0.041	0.051	0.047	0.053	0.059	0.058	0.067
$P^{\bar{x}}$ <	NS	0.05	0.001	0.01	0.01	0.001	0.001
% Change	+ 10	+20	+24	+26	+26	+51	+62
Compliance ml/cm H_2O							
Control	0.23	0.24	0.20	0.21	0.22	0.24	0.20
Exposure[a]	0.20	0.22	0.15	0.15	0.16	0.16	0.12
$\bar{x}$ (E-C)	-0.03	-0.02	-0.05	-0.06	-0.06	-0.08	-0.08
$S_{\bar{x}}$	0.015	0.016	0.014	0.017	0.012	0.017	0.019
$P^{\bar{x}}$ <	NS	NS	0.01	0.01	0.001	0.001	0.01
% Change	-13	-8	-25	-28	-27	-33	-40
Post Exposure[b]	0.21	0.23	0.17	0.17	0.17	0.18	0.14
$\bar{x}$ (P-C)	-0.02	-0.01	-0.03	-0.04	-0.05	-0.06	-0.06
$S_{\bar{x}}$	0.018	0.012	0.011	0.013	0.013	0.016	0.021
$P^{\bar{x}}$ <	NS	NS	0.02	0.01	0.01	0.01	0.01
% Change	-9	-4	-15	-19	-22	-25	-30

[a]"Exposure": Average of readings at 55 and 60 min.

[b]"Post Exposure": Average of readings at 25 and 30 min.

FIGURE 1 - DOSE-RESPONSE CURVES FOR SULFURIC ACID. THE NUMBERS BESIDE EACH POINT INDICATE THE NUMBER OF ANIMALS IN THAT GROUP.



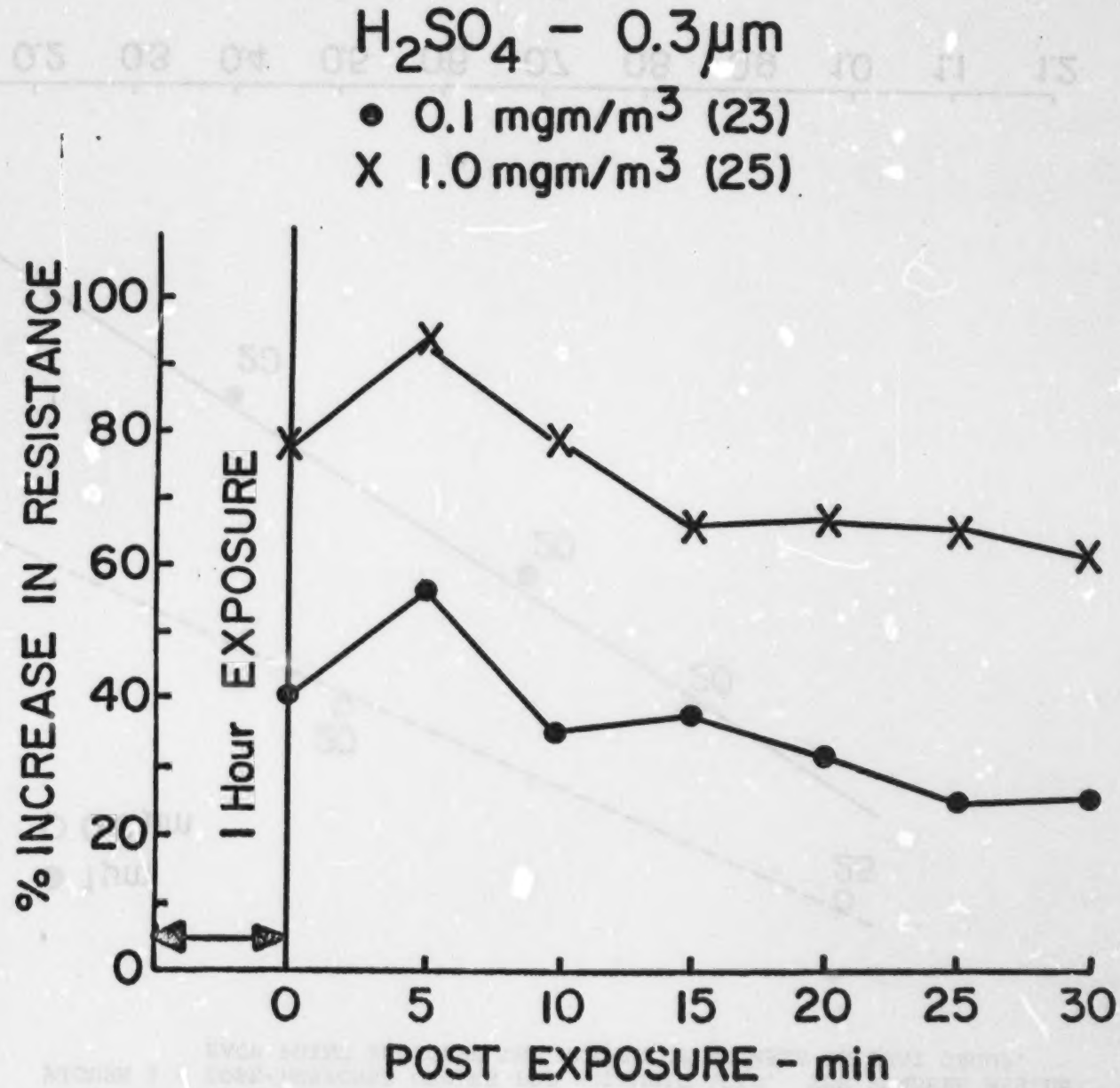


FIGURE 2 - POST-EXPOSURE CHANGES IN PULMONARY FLOW RESISTANCE PRODUCED BY 0.3 µm SULFURIC ACID. NUMBERS IN PARENTHESES ARE NUMBERS OF ANIMALS EXPOSED.

IV. Inorganic Sulfates

A. Methods of Aerosol Generation and Measurement

These aerosols were produced with Dautrebande D_{30} aerosol generators. These produce a heterogeneous aerosol of sub-micron size. The several models we have have slightly different characteristics in terms of mean size produced with a given concentration of solution. To vary size, we utilized this fact in combination with the use of 0.1, 0.3 and 1% solutions of the various salts. The size was measured by collecting a sample on a carbon-coated EM grid by electrostatic precipitation. The size was measured from electron micrographs.

The mass concentration of the sulfate salts was measured by various methods following collection on a Millipore filter. For the copper sulfate, copper was measured by atomic absorption. Ammonium sulfate and ammonium bisulfate were measured with an ammonium ion electrode, by direct weighing on a Cahn electrobalance or by increase in electrical conductivity.

Sulfur dioxide was generated by metering gas from a tank containing 0.1% sulfur dioxide in air. The concentration was measured by collecting a sample in dilute sulfuric acid-hydrogen peroxide solution and measuring the increase in conductivity. The collecting bubbler was preceded by a membrane filter to remove the aerosol, which would have also altered the conductivity.

The main air stream entering the exposure chamber was filtered to remove extraneous particles and dried. The relative humidity of the exposure chamber atmosphere was 50%.

B. Background

Data on the comparative toxicity of sulfate salts are of importance because sulfur dioxide in urban atmospheres is converted into particulate sulfur species. The rather meager existing toxicological data indicate that some, but not all, sulfate salts are respiratory irritants and that the irritant potency of a given sulfate increases with decreasing particle size.

Zinc ammonium sulfate causes an increase in pulmonary flow resistance in guinea pigs (Amdur and Corn, Amer. Indust. Hyg. Assoc. J. 24:326, 1963). Over the size range studied (0.29 to 1.4 μm, mean size by weight) the change in flow resistance increased as the particle size decreased. In cats, zinc ammonium sulfate also caused an increase in flow resistance and a decrease in pulmonary compliance (Nadel et al., Inhaled Particles and Vapors II:55, 1965). These changes were similar to the response to an aerosol of histamine, but were of lesser magnitude.

Zinc sulfate and ammonium sulfate (0.3 μm in size) produced an increase in flow resistance in guinea pigs (Amdur and Corn, 1963) as did ferric sulfate (Amdur and Underhill, Arch. Environ.

Health, 16:460, 1968). Neither ferrous sulfate nor manganous sulfate was irritant (Ibid.).

In the present studies we have examined the comparative irritant potency of ammonium sulfate, ammonium bisulfate, cupric sulfate and sodium sulfate. We have also determined whether or not these sulfate salts potentiate the response to low concentrations of sulfur dioxide.

C. Results

The effects of the four sulfate salts on resistance and compliance are presented in Table 2. In order that the various salts may be compared in irritant potency, the response is also expressed as percent change per microgram of sulfate.

Ammonium sulfate caused a statistically significant decrease in compliance at all the concentrations and particle sizes tested. Two of the exposures produced an increase in resistance and two did not. The resistance increase produced by the 0.3 μm particles at a concentration of 1 mg/m^3 was in good agreement with values found on a smaller group of animals in a previous study (Amdur and Corn, 1963). With the exception of the fact that there was a minimal response to the 0.2 μm particles, the response per μg of sulfate was greater as the particle size decreased.

Ammonium bisulfate was also a mild respiratory irritant. All the exposed groups showed a statistically significant increase in resistance and decrease in compliance. The degree of response per microgram of sulfate increased as the particle size decreased. As was the case with ammonium sulfate, the response was minimal to the 0.8 μm particles. An overall consideration of the data suggests that ammonium bisulfate is less irritant than ammonium sulfate. At the smallest size (0.13 μm) the percent change in resistance per μg of sulfate was 0.063 for the sulfate and 0.019 for the bisulfate; the corresponding values for compliance were -0.074 and -0.019. A similar pattern emerges when one compares the data for 0.3 μm ammonium sulfate with the data for 0.5 μm ammonium bisulfate. For what it's worth, these comparisons would tend to suggest that the response to ammonium sulfate is of the order of three times that to ammonium bisulfate.

The data for copper sulfate permit the direct comparison of two concentrations at similar particle sizes and of two particle sizes at similar concentrations. At a particle size of 0.1 μm a concentration of 0.4 mg/m^3 produced a slight but statistically

significant decrease in compliance. The slight decrease in resistance was not statistically significant. At a higher concentration of 2 mg/m^3 both an increase in resistance and a decrease in compliance were observed. Exposure to 2 mg/m^3 at a particle size of 0.3 μm produced a statistically significant change in resistance and compliance, but the response was slightly less than that produced by the smaller particles.

Sodium sulfate at a particle size of 0.1 μm produced no change in resistance. The slight decrease in compliance was not statistically significant.

Data for sulfur dioxide alone at a concentration of 0.3 ppm and for the combination of sulfur dioxide and the aerosols at a particle size of 0.1 μm at the lowest concentration used are presented in Table 3. In all exposures there was a statistically significant increase in resistance and decrease in compliance. Figure 3 compares the responses to the combined exposure with the sum of the responses to the sulfur dioxide and aerosol given alone. The combination of the copper sulfate and sulfur dioxide was more than additive. The response to the other combinations could be predicted on the basis of a simple additive response.

D. Discussion

Of the four sulfate salts examined, ammonium sulfate appeared to be the most irritant, followed by ammonium bisulfate, copper sulfate, and sodium sulfate. There is evidence that isolated guinea pig lung fragments release histamine when incubated with solutions of ammonium sulfate but not when incubated with solutions of sodium

sulfate (Charles and Menzel, Arch. Environ. Health 31:314, 1975). Intratracheal injections of ammonium sulfate solutions produced bronchoconstriction in isolated perfused lungs but sodium sulfate did not. Ammonium ions also increased the absorption of sulfate by the rat lung in vivo (Charles and Menzel, Res. Comm. Chem. Path. Pharm. 12:389, 1975). Sulfate removal was differentially enhanced and presumably there was increased flux by various associated cations across the mast cell where histamine is stored. Among the most active in this regard were ferric and zinc ions. In earlier work in our laboratory (Amdur and Corn, 1963; Amdur and Underhill, 1968), both ferric sulfate and zinc sulfate were found to be irritant. The pharmacological findings correlate well with the data obtained in our studies using mechanics of respiration as a means of determining relative irritant potency.

None of the sulfate salts tested in the present studies or in our earlier work are as irritant as sulfuric acid itself. The percent increase in resistance per µg of sulfate as sulfuric acid is 0.432 for 0.1 µm particles and 0.410 for 0.3 µm particles (Amdur, Proc. 4th Symp. Statistics and the Environment, pg. 48, 1976). Data for all the sulfates tested in these and earlier studies are available at the 0.3 µm size except sodium sulfate, which was only studied as 0.1 µm particles. If one assigns a value of 100 to the 0.410 obtained with sulfuric acid and relates the values for the sulfate salts to it, it is possible to rank these compounds for irritant potency as shown in Table 4. The ten-fold less irritant potency of ammonium sulfate as compared to sulfuric acid would fit the

observation made twenty years ago that neutralization with ammonia eliminated the lethality of sulfuric acid to guinea pigs (Pattle, Burgess and Cullumbine, J. Path. Bact. 72:219, 1956).

The irritant potency of the sulfate species varies so widely that the term "suspended sulfate" is toxicologically meaningless. The practical implication of this fact is that a better analytical measurement than "suspended sulfate" will be needed in research epidemiology before definitive data relating to health effects of particulate sulfur species are likely to emerge.

The range of particle size of the aerosols used in the present study (0.1 to 0.8 μm) was too narrow to demonstrate a rational relationship between particle size and degree of reponse. Such relationships were reported earlier for zinc ammonium sulfate over a size range of 0.3 to 1.4 μm (Amdur and Corn, 1963) and for sulfuric acid over a size range of 0.1 to 2.5 μm (Amdur, 1976). The data for any given compound in the present study, however, showed in general a greater degree of response at a smaller particle size. Overall, data from various studies in our laboratory indicate that measurement of mass median diameter would be much more meaningful than "respirable size" in attempts to assess the health effects of atmospheric aerosols.

Ammonium sulfate, ammonium bisulfate, and sodium sulfate in these studies did not potentiate the response to sulfur dioxide. These exposures were all performed at an exposure chamber relative humidity of 50%. There is evidence that increasing the relative humidity to 80% markedly increases the potentiating effect of sodium chloride (McJilton, Frank and Charlson, Science 182:503, 1973).

Whether or not increased relative humidity would effect these sulfate salts in a similar manner is not known.

It was previously reported that salts of manganese, vanadium, and ferrous iron were strong potentiators of the response to sulfur dioxide (Amdur and Underhill, 1968). At concentrations of 1.4 to 1.8 x 10^{-2} millimoles of metal per cubic meter the metal salts increased the response to 3 to 4 times that observed in exposures to sulfur dioxide alone. These metals were known to promote the conversion of sulfur dioxide to sulfuric acid. Under our exposure conditions about 10% conversion was found at sulfur dioxide concentrations of 0.2 ppm. When this amount of sulfuric acid was combined with 0.2 ppm sulfur dioxide, the response observed from sulfur dioxide and the metal aerosols was reproduced (Amdur, Amer. Indust. Hyg. Assoc. J. 35:589, 1974).

It had been reported that copper sulfate also promoted the conversion of sulfur dioxide to sulfuric acid (Cheng et al., Atmos. Environ. 5:987, 1971). On this basis, our observation that aerosols of copper sulfate potentiated the response to sulfur dioxide would have been predicted. It is interesting, however, to note that copper appears to be a more powerful potentiator of sulfur dioxide than the other metals. The concentration of copper used was 1.3 x 10^{-3} millimoles/m^3, or about one tenth of the concentration of the other metals. The response increased four-fold. A possible explanation for the greater effectiveness of copper may be provided by the observation made at the Center for Thermochemical studies at Brigham Young University that aerosols containing

copper can complex with sulfur dioxide in such a manner that the sulfur is relatively stable as S^{IV} and is to some extent protected from further oxidation (Hansen et al., Proc. Int. Conf. Environ. Sensing and Assessment, 1975). Their samples were from within a smelter or from the atmosphere when wind patterns brought material from known point sources. It has also been reported that sodium bisulfite is a much more powerful irritant than sulfur dioxide (Alarie, Wakisaka and Oka, Environ. Physiol. Biochem. 3:182, 1973). These data raise the interesting speculation that perhaps the copper potentiation is mediated by the formation of a sulfite or bisulfite complex rather than by the formation of sulfuric acid.

Overall, these data emphasize the importance of analysis of specific trace metal content of atmospheric aerosols in studies attempting to unravel the complexities of the health effects of the sulfur oxides-particulate pollution complex.

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Table 2

Respiratory Response to Sulfate Salts

Sulfate Salt	$(NH_4)_2 SO_4$				NH_4HSO_4			$CuSO_4$			Na_2SO_4
MMD - µm	0.13	0.20	0.30	0.81	0.13	0.52	0.77	0.11	0.13	0.33	0.11
Salt - mg/m³	0.50 ± 0.16	2.14 ± 0.23	1.02 ± 0.11	9.54 ± 0.94	0.93 ± 0.09	2.60 ± 0.29	10.98 ± 1.64	0.43 ± 0.17	2.05 ± 0.28	2.41 ± 0.31	0.90 ± 0.11
µg SO_4/m³	363	1553	726	6926	775	2168	9157	257	1232	1448	608
No. of Animals	10	10	10	10	19	10	10	23	30	35	10
Resistance cm H_2O/ml/sec											
control	0.43	0.53	0.65	0.60	0.60	0.87	0.59	0.44	0.44	0.49	0.66
exposure	0.53	0.51	0.83	0.60	0.69	1.11	0.73	0.48	0.55	0.56	0.67
difference	+ 0.10	-0.02	+ 0.18	0	+ 0.09	+ 0.24	+0.14	+0.04	+0.11	+0.07	+0.01
$S_{\bar{x}}$ [a]	0.041	0.043	0.052	-	0.035	0.059	0.043	0.021	0.032	0.021	-
$p <$ [b]	0.05	N.S.	0.01	-	0.02	0.01	0.02	NS	0.01	0.01	NS
% change	+ 23	-4	+ 29	0	+ 15	+27.5	+23	+9	+25	+14	+2
% change/µg SO_4	0.063	-	0.039	0	0.019	0.013	0.002	-	0.020	0.009	0.003
Compliance ml/cm H_2O											
control	0.29	0.23	0.30	0.26	0.26	0.23	0.26	0.27	0.27	0.27	0.26
exposure	0.21	0.20	0.23	0.23	0.22	0.16	0.21	0.24	0.23	0.24	0.24
difference	-0.08	-0.03	-0.07	-0.03	-0.04	-0.07	-0.05	-0.03	-0.04	-0.03	-0.02
$S_{\bar{x}}$ [a]	0.019	0.013	0.012	0.013	0.011	0.013	0.011	0.012	0.008	0.006	0.017
$p <$ [b]	0.01	0.05	0.001	0.05	0.01	0.001	0.01	0.02	0.001	0.001	NS
% change	-27	-13	-23	-12	-15	-30	-19	-11	-15	-11	-7
% change/µg SO_4	-0.074	-0.008	-0.032	-0.002	-0.019	-0.014	-0.002	-0.043	0.012	-0.008	-0.011

[a] Standard Error of Difference

[b] Students paired test

Table 3

Response to 0.1 µm Sulfate Salts and SO_2

SO_2 - ppm	0.32	0.30	0.32	0.36	0.31
Sulfate Salt	-	$(NH_4)_2SO_4$	NH_4HSO_4	$CuSO_4$	Na_2SO_4
Conc. mg/m^3	-	0.5	0.9	0.4	0.9
Number of Animals	10	10	10	10	10
Resistance - $CmH_2O/ml/sec$					
control	0.62	0.50	0.61	0.49	0.53
exposure	0.69	0.66	0.74	0.78	0.59
difference	+0.07	+0.16	+0.13	+0.29	+0.06
$S\bar{x}$	0.023	0.051	0.047	0.067	0.024
P <	0.02	0.02	0.05	0.01	0.05
% change	+12	+31	+ 21	+59	+11
Compliance - ml/CmH_2O					
control	0.22	0.23	0.28	0.30	0.31
exposure	0.20	0.18	0.21	0.19	0.27
difference	0.02	-0.05	-0.07	-0.11	-0.04
$S\bar{x}$	0.012	0.020	0.019	0.021	0.017
P <	NS	0.05	0.01	0.001	0.05
% change	-10	-22	-25	-37	-13

Table 4

Relative Irritant Potency of Sulfates

Sulfuric Acid	100
Zinc Ammonium Sulfate[a]	33
Ferric Sulfate[b]	26
Zinc Sulfate[a]	19
Ammonium Sulfate	10
Ammonium bisulfate	3
Cupric Sulfate	2
Ferrous Sulfate	0.7
Sodium Sulfate[c]	0.7
Manganous Sulfate[b]	-0.9[d]

[a] Data of Amdur and Corn, 1963

[b] Data of Amdur and Underhill, 1968

[c] Particle size 0.1 μm

[d] Resistance decreased; change N.S.

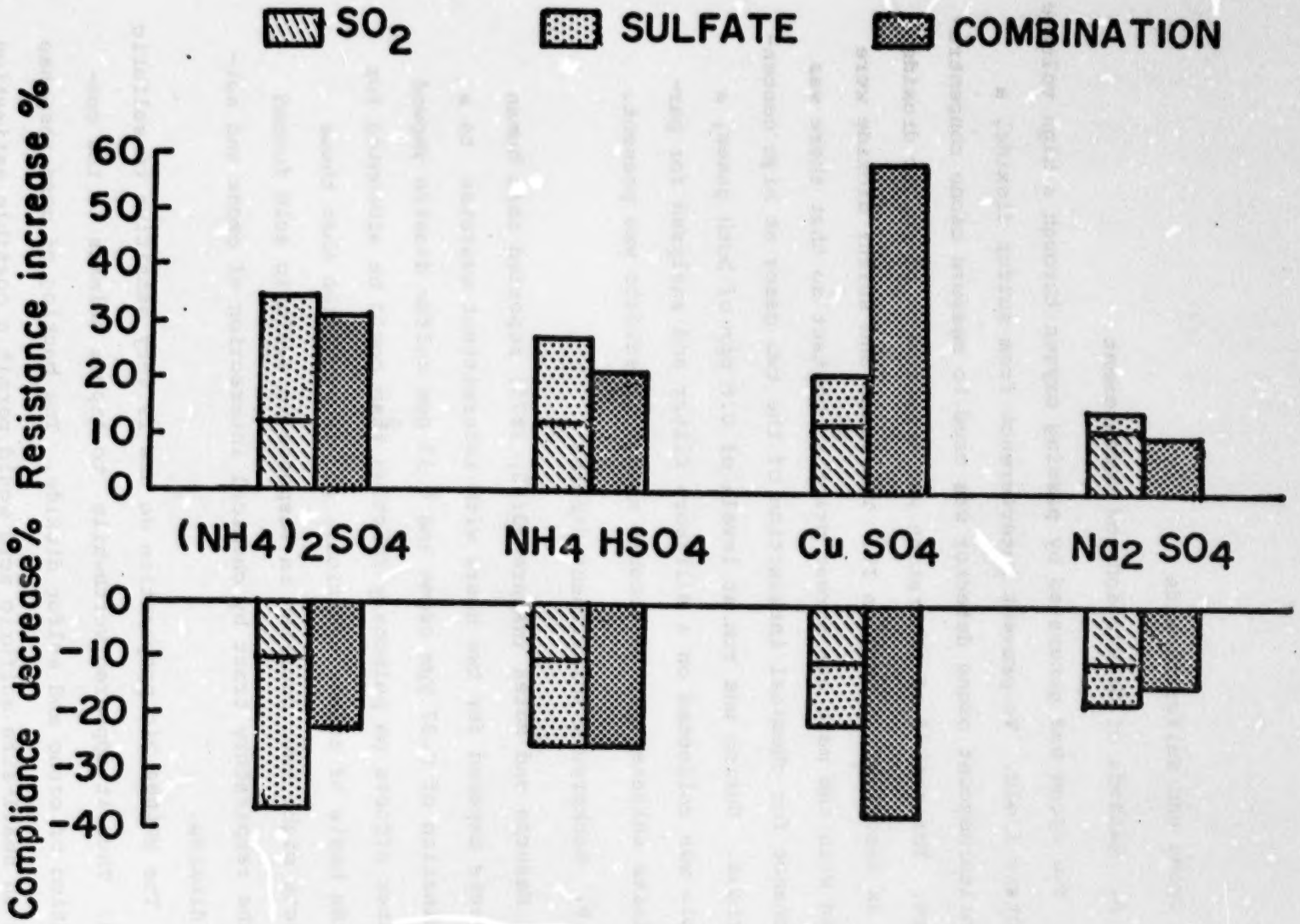

FIGURE 3 - COMPARISON OF RESPONSE TO COMBINATION OF SO_2 AND SULFATES WITH SUM OF THE RESPONSES TO EACH GIVEN ALONE.

V. Ozone and Sulfur Dioxide

A. Methods of Generation and Measurement

The ozone was generated by passing oxygen through a high voltage electric field. To prevent interference from sulfur dioxide, a chemiluminescent ozone detector was used to measure ozone concentrations. The method of generation and measurement of sulfur dioxide was as described in Section IV. The ozone and sulfur dioxide were mixed with the main air stream prior to contact so that there was no chance for chemical interaction of the two gases at high concentrations. During one run at levels of 0.8 ppm of both gases, a sample was collected on a Millipore filter and analysed for particluate sulfate. No detectable amount of sulfate was present.

B. Background for Present Studies

Hazucha and Bates (Nature 33:50, 1975) reported that human subjects exposed for two hours with intermittent exercise to a combination of 0.37 ppm ozone and 0.37 ppm sulfur dioxide showed greater effects on pulmonary function than could be accounted for on the basis of simple addition. It was suggested that these effects might have occurred in response to sulfuric acid formed in the respiratory tract by chemical interaction of ozone and sulfur dioxide.

The guinea pig preparation we use is very sensitive to sulfuric acid. Thus it appeared worthwhile to expose animals to the combination of ozone and sulfur dioxide. The backlog of dose-response data on small-size sulfuric acid would permit a possible estimation

of the amount formed. Our initial protocol was to expose groups of animals for two hours to 0.2, 0.4, or 0.8 ppm of each gas or to combinations of the two at equal concentrations. On the basis of the results of the exposures to the two higher levels, the planned experiments on the combination of the lowest concentration were not carried out.

C. Results

The response to ozone alone is shown in Table 5. As had been previously observed, ozone concentrations of this order of magnitude do not alter pulmonary flow resistance. The two higher concentrations produced a decrease in compliance of 22-24% below control values after one hour of exposure and 28-29% below control values by the end of the two-hour exposure. The post-exposure period of 30 minutes was not long enough for complete reversal. Earlier work showed that control values would be reached within two hours after exposure. The decrease in compliance resulted in a decrease in the time constant of the lungs and was accompanied by an increase in respiratory frequency. At the lowest concentration the increase in frequency was the only statistically significant change produced by the two-hour exposure.

The response to the exposure to sulfur dioxide alone is shown in Table 6. In these particular groups of animals the response was minimal and no statistically significant alterations in respiration were produced. There was no progressive increase in flow-resistance produced by the extension of the exposure time to two hours.

The response to the combined exposures to 0.4 and 0.8 ppm of each gas are shown in Table 7. The responses observed are essentially the same as those produced by the exposure to these concentrations of ozone alone. This response pattern is not typical of the response pattern produced by sulfuric acid.

D. Discussion

The results of the exposure to the combination of ozone and sulfur dioxide do not indicate a synergism between the two gases under the exposure conditions prevailing in these experiments. The response typical of ozone exposure, i.e. an increase in frequency, a decrease in compliance, minimal change in resistance and a decrease in the time constant, was observed in response to the combination of ozone and sulfur dioxide. This pattern of response is not similar to the changes produced in the quinea pig by exposure to sulfuric acid.

Since this work was done, Bill and Hackney at Rancho Los Amigos in Los Angeles have done further exposures of human subjects to combinations of ozone and sulfur dioxide. Their overall finding was that the effect was much less dramatic than that originally observed by Hazucha and Bates in Montreal. Together the two groups explored the various possible reasons for the observed difference. The most likely explanation appears to be the fact that the conditions in the Montreal exposure chamber led to the production in the exposure atmosphere of perhaps up to 200 $\mu g/m^3$ of acid sulfate, most likely sulfuric acid (Bill et al., Am. Indust. Hyg. Assoc. J., in press). The negative results obtained in our studies, in which no

sulfate was present in the chamber, suggest that there was no interaction of the ozone and sulfur dioxide after inhalation, as was originally postulated, at least in the guinea pig lung.

Table 5

Response to Ozone

PPM		0.2	0.4	0.8
Number of Animals		10	10	10
Resistance cm H_2O/ml/sec	Control	0.74	0.67	0.60
	1 hr	0.62	0.65	0.50
	2 hr	0.56	0.67	0.44
	Post Exp.	0.56	0.50	0.47
Compliance ml/cm H_2O	Control	0.25	0.25	0.27
	1 hr	0.23	0.19*	0.21*
	2 hr	0.22	0.18*	0.19*
	Post Exp.	0.22	0.20*	0.21*
R x C sec	Control	0.185	0.167	0.162
	1 hr	0.143	0.123	0.105*
	2 hr	0.123	0.120	0.084*
	Post Exp.	0.123	0.100	0.099*
Frequency breaths/min	Control	80	84	86
	1 hr	89	93	97*
	2 hr	94*	99*	115*
	Post Exp.	93*	96*	113*
Tidal Volume ml	Control	2.3	2.4	2.6
	1 hr	2.3	2.2	2.2
	2 hr	2.3	2.0	1.8
	Post Exp.	2.1	2.3	2.0
Minute Volume ml	Control	184	201	223
	1 hr	205	205	213
	2 hr	216	198	207
	Post Exp.	195	221	226

*Statistically significant: $p < 0.05$ or better.

Table 6

Response to Sulfur Dioxide

PPM		0.2	0.4	0.8
Number of Animals		10	10	10
Resistance cm H_2O/ml/sec	Control	0.62	0.59	0.62
	1 hr	0.64	0.63	0.66
	2 hr	0.59	0.57	0.64
	Post Exp.	0.58	0.56	0.60
Compliance ml/cm H_2O	Control	0.24	0.20	0.23
	1 hr	0.21	0.20	0.23
	2 hr	0.21	0.19	0.24
	Post Exp.	0.20	0.18	0.22
R x C sec	Control	0.149	0.118	0.143
	1 hr	0.134	0.126	0.152
	2 hr	0.124	0.108	0.154
	Post Exp.	0.116	0.101	0.132
Frequency breaths/min	Control	87	89	78
	1 hr	93	95	80
	2 hr	94	92	76
	Post Exp.	89	94	74
Tidal Volume ml	Control	2.3	2.0	2.1
	1 hr	2.2	2.1	2.3
	2 hr	2.3	2.0	2.2
	Post Exp.	2.1	2.1	2.1
Minute Volume ml	Control	200	178	169
	1 hr	204	199	184
	2 hr	216	184	167
	Post Exp.	187	197	155

Table 7

Response to Combination of Ozone
and Sulfur Dioxide

PPM		0.4	0.8
Number of Animals		10	10
Resistance	Control	0.42	0.55
cm H_2O/ml/sec	1 hr	0.40	0.56
	2 hr	0.33	0.42
	Post Exp.	0.31	0.37
Compliance	Control	0.27	0.28
	1 hr	0.23	0.23
	2 hr	0.22*	0.21*
	Post Exp.	0.28	0.24
R x C	Control	0.113	0.154
sec	1 hr	0.092	0.129
	2 hr	0.072*	0.088*
	Post Exp.	0.087*	0.089*
Frequency	Control	91	71
breaths/min	1 hr	104*	89*
	2 hr	108*	88*
	Post Exp.	111*	99*
Tidal Volume	Control	2.6	2.6
ml	1 hr	2.2	2.4
	2 hr	2.1	2.1
	Post Exp.	1.9	2.1
Minute Volume	Control	237	184
ml	1 hr	229	214
	2 hr	227	185
	Post Exp.	211	208

*Statistically significant: $p < 0.05$ or better.

VI. Oil Mists and Sulfur Dioxide

This work is described in detail by Daniel L. Costa in a thesis: The Physical and Physiological Effects of Oil Mists and Sulfur Dioxide (Harvard School of Public Health, May, 1977). Two manuscripts for publication are currently being prepared from this material.

When simultaneous exposures were made to 1 or 10 ppm sulfur dioxide and 10 mg/m^3 motor oil, the irritant effects of sulfur dioxide (resistance increase) were antagonized. Mineral oil (medicinal grade napthene oil) did not protect against sulfur dioxide. When either the detergent or the dispersant component of the "additive package" used in the motor oil were added to mineral oil, partial protection was obtained. The addition of both of these components to mineral oil essentially reproduced the protection resulting from the motor oil. We were unable to elicit true cooperation from the manufacturers due to the proprietary nature of their product. Our attempts to negotiate with them bore more resemblance to a game of twenty questions than to a scientific inquiry.

Neither motor oil nor medicinal mineral oil protected animals from the irritant effects of formaldehyde, suggesting that the protection observed with the motor oil was specific for sulfur dioxide and not a general protection against irritant action per se.

One curious finding, which was not included in the thesis write-up, was the fact that the addition of α-tocopherol to the medicinal mineral oil would protect against the irritant action of sulfur dioxide. We currently have no rational explanation to offer

for this. It is probably worth further work to determine whether it was a local effect in the lung or would also be observed if vitamin E were administered by more usual routes.

VII. Preliminary work on Sulfites

The finding by the group at Brigham Young University that in the presence of some trace metals, sulfur remains as S^{IV} rather than being oxidized to S^{VI} points up the need for toxicological work on sulfites and bisulfites.

Alarie et al. (Environ. Physiol. Biochem. 3:182, 1973) reported that sodium bisulfite was more irritating than sulfur dioxide and that sodium sulfite was not irritant. They were using reduction of respiratory frequency in mice as the criterion of irritant response. The concentrations are all reported as SO_2 equivalents.

If one aerosolizes a solution of sodium bisulfite, one ends up by generating sulfur dioxide, with no sulfur in a particle mode. Alarie used a glycol to stabilize the aerosol phase, but the paper does not define how much sulfur was in the aerosol phase and how much was present as sulfur dioxide gas. A phone call indicated that as they had not used a filter in their sampling system, no effort had been made to determine this factor. We tried their system, using the glycol. With the bisulfite, some was indeed present on the filter, but some was also present as sulfur dioxide gas (unless of course it came off the filter rather than being present in the chamber atmosphere).

The system had other disadvantages, as there is evidence that some liquid aerosols capable of dissolving sulfur dioxide will cause potentiation of response to the gas. In order to properly cope with this possibility, we would have had to run a separate series with the glycol plus sulfur dioxide gas. Overall, the system seemed too inoptimum to spend further time on at this moment. We also had a problem with an infection in our supply colony of guinea pigs. These factors, combined with problems of moving myself and my lab from Harvard to M.I.T., led me to throw in the sponge on this project for the time being. I plan to return to the problem of S^{IV}-aerosol complexes, but not via this particular generation system.